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|---|---|---|---|
| (51) International Patent Classification 6 : C07D 498/18, A61K 31/445 | A1 | (11) International Publication Number: | WO 98/09972 |
| | | (43) International Publication Date: | 12 March 1998 (12.03.98) |

(21) International Application Number: PCT/US97/15438

(22) International Filing Date: 2 September 1997 (02.09.97)

(30) Priority Data:
08/709,318 9 September 1996 (09.09.96) US

(71) Applicant: AMERICAN HOME PRODUCTS CORPORATION [US/US]; Five Giralda Farms, Madison, NJ 07940-0874 (US).

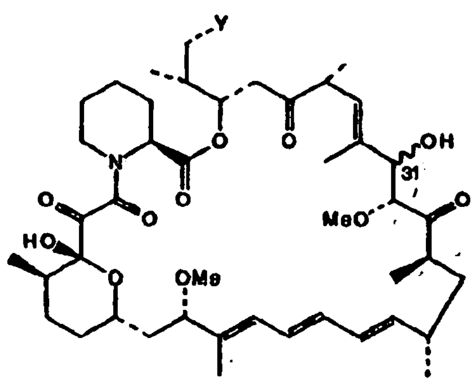
(72) Inventors: GRINFIELD, Alexander, Alesksey; 122 Harris Road, Princeton Junction, NJ 08550 (US). HU, David, Cheng; 160 Mira Street, Foster City, CA 94404 (US). CAUFIELD, Craig, Eugene; 28 Cranbury Road, Princeton Junction, NJ 08550 (US).

(74) Agents: ALICE, Ronald, W.; American Home Products Corporation, One Campus Drive, Parsippany, NJ 07054 (US) et al.

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

**Published**
*With international search report.*
*Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.*

(54) Title: RAPAMYCIN DERIVATIVES WITH UNNATURAL STEREOCHEMISTRIES



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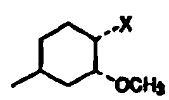
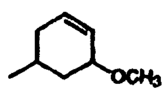


(a)



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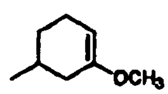

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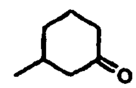

(d)

(57) Abstract

This invention relates to compounds which possess immunosuppressive and/or anti-tumor and/or antiinflammatory activity *in vivo* and/or inhibit thymocyte proliferation *in vitro*. These compounds are therefore useful in the treatment of transplantation rejection, autoimmune diseases such as lupus, rheumatoid arthritis, diabetes mellitus, multiple sclerosis and in the treatment of *Candida albicans* infections and also in treatment of diseases of inflammation. These compounds as represented by formula (I) where Y is a group selected from the groups (a), (b), (c) or (d), wherein X is selected from hydroxy, $-OR^1$, $-SO_2Ar$, $-SO_2R^1$, $N_3$, -OAr, -NH(C=O)Ar, $-NH(C=O)R^1$, $-NH(C=O)NR^2R^3$, -NHCN, I, Cl, F, Br, -SCN, or 1,2,3-triazole optionally substituted with methoxycarbonyl and $R^1$-$R^5$ are as defined herein.

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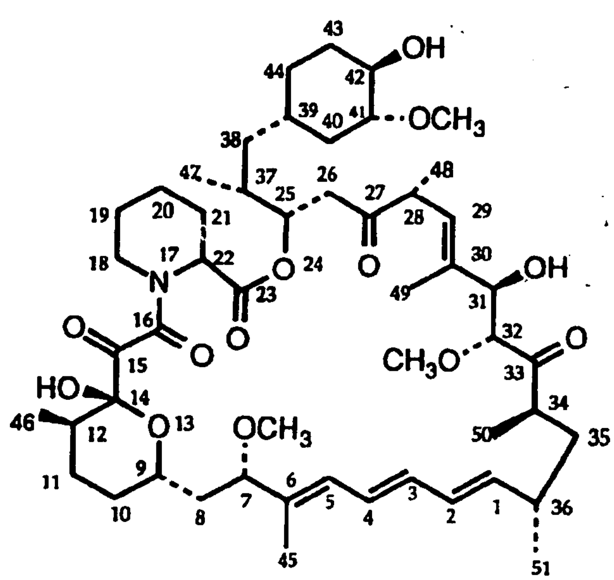
# RAPAMYCIN DERIVATIVES WITH UNNATURAL STEREOCHEMISTRIES

This application claims benefit of priority of US provisional application number 60/025,695 filed on September 9, 1996.

This invention relates to compounds of formula I below or pharmaceutically acceptable salts thereof which possess immunosuppressive and/or anti tumor and/or antiinflammatory activity *in vivo* and/or inhibit thymocyte proliferation *in vitro*. These compounds are therefore useful in the treatment of transplantation rejection, autoimmune diseases such as lupus, rheumatoid arthritis, diabetes mellitus, multiple sclerosis and in the treatment of *Candida albicans* infections, the treatment of diseases of inflammation, treatment of hyperproliferative vascular disease (restenosis) and in the treatment of certain human tumors.

## BACKGROUND OF THE INVENTION

Rapamycin is a macrocyclic triene antibiotic produced by Streptomyces hygroscopicus, which was found to have antifungal activity, particularly against Candida albicans, both in vitro and in vivo [C. Vezina et al., J. Antibiot. 28, 721 (1975); S.N. Seghal et al., J. Antibiot. 28, 727 (1975); H. A. Baker et al., J. Antibiot. 31, 539 (1978); U.S. Patent 3,929,992; and U.S. Patent 3,993,749].



**Rapamycin**
(Positions numbered according to *Chemical Abstracts*)



SUBSTITUTE SHEET (RULE 26)

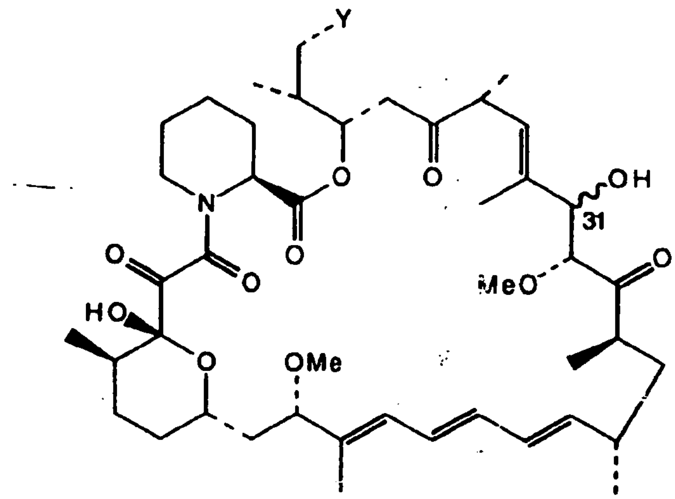
Rapamycin alone (U.S. Patent 4,885,171) or in combination with picibanil (U.S. Patent 4,401,653) has been shown to have antitumor activity against transplantable carcinogenic tumors in mice. R. Martel et al. [Can. J. Physiol. Pharmacol. 55, 48 (1977)] disclosed that rapamycin is effective in the experimental allergic encephalomyelitis model, a model for multiple sclerosis; in the adjuvant arthritis model, a model for rheumatoid arthritis; and effectively inhibited the formation of IgE-like antibodies.

The immunosuppressive effects of rapamycin have been disclosed in FASEB 3, 3411 (1989). Rapamycin has been shown to be effective in inhibiting transplant rejection (U.S. Patent 5,100,899). Cyclosporin A and FK-506, other macrocyclic molecules, also have been shown to be effective as immunosuppressive agents, therefore useful in preventing transplant rejection [FASEB 3, 3411 (1989); FASEB 3, 5256 (1989); and R. Y. Calne et al., Lancet 1183 (1978)]. U. S. patent 5,321,009 discloses a method of prophylactically preventing the onset, preventing the development, and arresting the progression of insulin-dependent diabetes mellitus by administration of rapamycin. U. S. patent 5,288,711 discloses a method of preventing or treating hyperproliferative vascular disease by administration of a combination of rapamycin and heparin. U. S. patent 5,286,730 discloses a method of treating immunoinflammatory disease by treatment with rapamycin alone or in combination with cyclosporin A. U. S. patent 5,286,731 provides a method of treating immunoinflammatory bowel disease by administration of rapamycin alone or in combination with cyclosporin A. Various structural features of rapamycin have been modified in efforts to increase the potency or specificity of pharmacological action. For instance, a number of U. S. patents disclose compounds where one or more of the hydroxy groups having normal stereochemistry at positions 14, 31, and 42 have been converted into acyl esters, sulfonyl esters, and carbamates. U. S. patent 5,023,263 discloses 42-oxo rapamycin. U. S. patent 5,258,389 discloses 31 and/or 42 O-alkyl, O-aryl, O-alkenyl, and O-alkynyl ethers of rapamycin having normal stereochemistry at the 42 position. The PCT published application WO 94/09010 discloses 31 and/or 42 O-alkylated rapamycin analogs wherein the keto groups at positions 15 and 33 may be reduced to a hydroxyl group or a methylene group.

## SUMMARY OF THE INVENTION

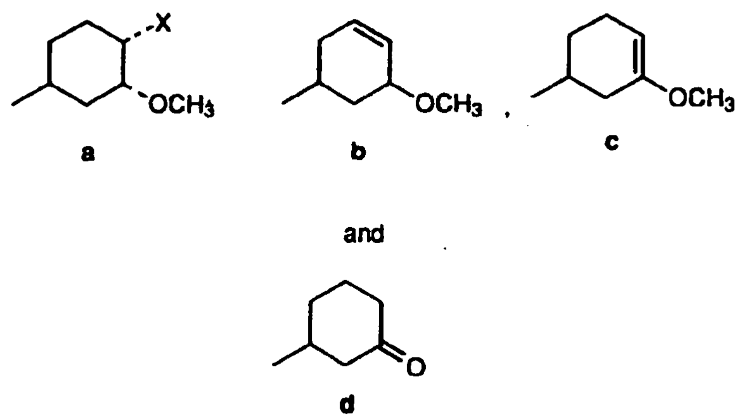
The rapamycin compounds of this invention are either epimeric (S-configuration) with rapamycin at position 42 alone or positions 31 and 42, or derived from reactions to produce 42-dehydroxy-42-epi-substituted rapamycin analogs. These rapamycin analogs are represented by formula I below and may be further designated as formulas Ia, Ib, Ic or Id depending on the group Y. The compounds of formulas Ib and Ic result from

elimination reactions competing with nucleophilic substitutions and the formula Id compound results from rearrangement of the formula Ic compound.



I

In formula I above, Y is a group selected from the groups a, b, c, or d below.



In group **a** above, X is selected from hydroxy, $-OR^1$, $-SO_2Ar$, $-SO_2R^1$, $N_3$, -OAr, -NH(C=O)Ar, $-NH(C=O)R^1$, $-NH(C=O)NR^2R^3$, -NHCN, I, Cl, F, Br, -SCN, or 1,2,3-triazole optionally substituted with methoxycarbonyl, where the stereochemical configuration at position 42 is epimeric with naturally occurring rapamycin.

$R^1$ is $C_1$ to $C_{10}$ alkyl, cycloalkyl of 3 to 10 carbon atoms, $-(CH_2)_nNHR^2$, piperidinyl, pyrrolidinyl, piperazinyl, $-(CH_2)_nAr$, $-CH_2CH(OR^4)CH_2OR^5$, or $-CH_2$-1,2:3,4-diisopropylidenegalactose. $R^2$ and $R^3$ are independently $C_1$ to $C_{10}$ alkyl, Ar, H, or $-(CH_2)_nAr$. $R^4$ and $R^5$ are independently H, $C_1$ to $C_{10}$ alkyl, $-(CH_2)_nAr$, or $R^4$ and $R^5$ together form isopropylidene.

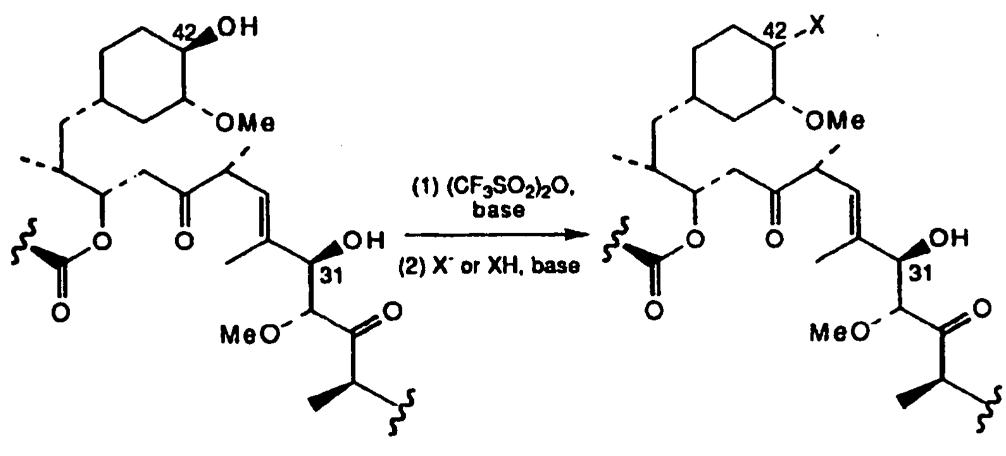
In the above definitions of $R^1$, $R^2$, $R^3$, $R^4$ and $R^5$, n is from 1-10 and Ar is independently selected from phenyl, naphthyl, pyridyl, quinolyl, indolyl, imidazolyl, triazolyl, tetrazolyl, furanyl, and thiophenyl, optionally substituted by one or two substituents selected independently from F, Cl, Br, I, $NO_2$, OH, $C_1$–$C_{10}$ alkyl, $C_1$–$C_{10}$ alkoxy, or hydroxymethyl, or aryl is 3,4-methylenedioxyphenyl. The term "$C_1$ to $C_{10}$" alkyl encompasses straight as well as branched-chain hydrocarbons. This invention also encompasses the pharmaceutically acceptable acid addition salts when they can be formed with pharmaceutically acceptable inorganic or organic acids such as hydrochloric, sulfuric, phosphoric, mono or dibasic ammonium phosphoric, acetic, fumaric, maleic, malic, citric, succinic, and tartaric acids.

The compounds of this invention exhibit immunosuppressive and/or antifungal and/or antitumor and/or antiinflammatory activity *in vivo* and/or inhibit thymocyte proliferation *in vitro* and are therefore useful in the treatment or inhibition of organ or tissue transplantation rejection or host vs. graft disease, proliferative diseases such as restenosis following angioplasty procedures, autoimmune diseases such as lupus, rheumatoid arthritis, diabetes mellitus, and multiple sclerosis; fungal infections, and diseases of inflammation such as psoriasis, exzema, seborrhea, inflammatory bowel disease and pulmonary inflammation such as asthma, chronic obstructive pulmonary disease, emphysema, bronchitis and the like.

Compounds of this invention also inhibit in vitro in submicromolar concentrations cell growth of certain tumor cells, most notably prostate (PC-3), breast (T47D, SKBR-3) and ovarian (A 2780S) cells, and therefore are useful in the treatment of these and other tumors.

**Detailed Description of the Invention**

The compounds of the invention where Y is the 3-methoxy-4-epi-substituted cyclohexyl ring a are prepared by standard literature procedures as outlined below.




Rapamycin I

Invention compounds where Y is **b**, **c**, or **d** in formula I above may be obtained as side products in the reaction shown above. The amounts of these side products obtained from the reaction vary as to the nature of the nucleophile $X^-$ or XH used. The following synthetic examples are included to illustrate the synthetic methods outlined above and are not intended to limit this disclosure in any way. The reagents used are either commercially available or readily prepared by those skilled in the art of organic synthesis.

Example 1.

**Rapamycin-42-Triflate**

To a solution of rapamycin (0.914 g, 1.00 mmol) in dichloromethane (15.0 mL) in a round bottom flame dried flask (25 mL) equipped with a magnetic stirrer at room temperature was added 2,6-di-t-butyl-4-methylpyridine (0.60 g, 2.92 mmol). The reaction mixture was degassed, purged with nitrogen, and cooled to 0 °C. To the solution was added dropwise trifluoromethanesulfonic anhydride (0.170 mL, 0.282 g, 1.00 mmol). over a period of 5 min. The solution became a suspension. The reaction was stirred at 0 °C for 30 min then warmed up to room temperature. TLC analysis (silica gel, eluent: EtOAc/hexane) showed completion of the reaction.

**General Procedures for the Preparation of 42-substituted Derivatives of Rapamycin via Nucleophilic Substitution**

The following general procedures designated as Method A and Method B were used in preparing the invention compound and the method used is indicated in the specific example.

**Method A**

To the solution of rapamycin-42-triflate prepared from 0.914 g (1 mmol) of rapamycin was added at room temperature (unless otherwise noted) the appropriate nucleophile (5 - 10 eq depending on its nucleophilicity and relative basicity) was added. The mixture was stirred between 4 and 96 h monitoring the extent of the reaction by TLC. When the desired conversion had been achieved the reaction was quenched with saturated aqueous $NaHCO_3$ and the organic and aqueous layers were separated. The aqueous layer was extracted three times with ethyl acetate. The organic layers were combined, washed with brine, and dried over sodium sulfate. The solution was filtered and concentrated *in vacuo* to afford product. Pure products were isolated by HPLC (normal phase, Dynamax 2" silica column, eluent: EtOAc/hexane, 20 mL/min; reversed phase,

Dynamax 2" C-18 column, eluent: MeCN/water, 20 mL/min). Spectroscopic analyses were used to confirm the structures.

**Method B**

A solution of rapamycin-42-triflate prepared from 0.914 g (1 mmol) of rapamycin was cooled to -20 °C, kept at this temperature for 30 min, and filtered under nitrogen though a sintered glass filter to remove precipitated salts. The precipitate was washed twice with 2 mL of dichloromethane cooled to -20 °C. The combined clear solution was allowed to reach room temperature and the appropriate nucleophile (5 - 10 eq depending on its nucleophilicity and relative basicity) was added. The mixture was stirred between 4 and 96 h monitoring the extent of the reaction by TLC. When the desired conversion had been achieved the reaction was quenched with saturated aqueous $NaHCO_3$ and the organic and aqueous layers were separated. The aqueous layer was extracted three times with ethyl acetate. The organic layers were combined washed with brine and dried over sodium sulfate. The solution was filtered and concentrated *in vacuo* to afford product. Pure products were isolated by HPLC (normal phase, Dynamax 2" silica column, eluent: EtOAc/hexane, 20 mL/min; reversed phase, Dynamax 2" C-18 column, eluent: MeCN/water, 20 mL/min). Spectroscopic analyses were used to confirm the structures.

Example 2.

<u>42-Deoxy-42(S-)iodo-rapamycin</u>

Method A. Nucleophile used: 670 mg (5 mmol) of lithium iodide. The reaction progress was monitored by TLC. Separation technique employed: Dynamax 2" silica column, eluent 60 % EtOAc/hexane, 20 mL/min; Yield of product: 410 mg (40 %) Spectral data follows: $^1$H NMR (400 MHz, DMSO-$d_6$) δ 6.0 - 6.5 (m, 4 H, vinylic), 3.20 (s, 3 H, -O*Me*), 3.14 (s, 3 H, OMe), 3.03 (s, 3 H, OMe); IR (KBr, cm$^{-1}$) 3420, 2940, 1740, 1645, 1450; MS (neg. FAB), 1023 (M-), 897 (M-I) , 590;

| | |
|---|---|
| Anal calcd. for $C_{51}H_{78}NO_{12}I$: | C 59.82, H 7.68, N 1.37 |
| Found: | C 59.93, H 7.78, N 1.22 |

Example 3.

**42-Deoxy-42(S)-azido-rapamycin**

Method A at 35 °C. Nucleophile used: 455 mg (7 mmol) of sodium azide. Reaction time: 24 h; Separation technique employed: Dynamax 2" silica column, eluent 40 % EtOAc/hexane, 20 mL/min. Yield of product: 340 mg (36 %).
Spectral data follows: $^{1}H$ NMR (400 MHz, DMSO-$d_6$) $\delta$ 6.0 - 6.5 (m, 4 H, vinylic), 4.2 (m, 1 H, -$CHN_3$), 3.26 (s, 3 H, -O*Me*), 3( s, 3 H, -O*Me*), 3.0 (s, 3 H, -O*Me*); IR (KBr, $cm^{-1}$) 3440, 2930, 2100, 1735, 1645, 1450; MS (neg. FAB) 938 (M-), 590, 346;

| | |
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| Anal calcd. for $C_{51}H_{78}N_4O_{12}$: | C 65.22, H 8.37, N 5.97 |
| Found: | C 65.47, H 8.30, N 5.12 |

Example 4.

**42-Deoxy-42(S)-O-(2,2-Dimethyl-[1,3]-dioxolan-4-ylmethyl)rapamycin**

Method A. Nucleophile used: 670 mg (5 mmol) of 1,2-isopropylideneglycerol. Reaction time: 24 h; Separation technique employed: Dynamax 2" silica column, eluent 40 % EtOAc/hexane, 20 mL/min.; Yield of productas the monohyrdate: 185 mg (18 %).

Spectral data follows: $^{1}H$ NMR (400 MHz, DMSO-$d_6$) $\delta$ 6.0 - 6.5 (m, 4 H, vinylic), 3.5 - 4.0 (m, 5H), 3.25 (s, 3 H, -O*Me*), 3.14 (s, 3 H, -O*Me*), 3.03 (s, 3 H, -O*Me*), 1.28 (s, 3 H, *Me*C-OR), 1. 24 (s, 3 H, *Me*C-OR); IR (KBr, $cm^{-1}$) 3430, 2940, 1740, 1720, 1640, 1460; MS (neg. FAB) 1027 (M-), 590, 435;

| | |
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| Anal calcd. for $C_{57}H_{89}N_4O_{15} \cdot H_2O$: | C 65.39, H 8.70, N 1.34, |
| Found: | C 65.04, H 8.39, N 1.34 |

Example 5.

**42-Deoxy-42(S-)benzenesulfonyl-rapamycin**

Method A. Nucleophile used: 980 mg (6 mmol) of sodium phenylsulfinate; Reaction time: 24 h.; Separation technique employed: Dynamax 2" silica column, eluent 50 % EtOAc/hexane, 20 mL/min.; Yield of product as the monohydrate: 342 mg (33 %).

Spectral data follows: $^1$H NMR (400 MHz, DMSO-$d_6$) δ 7.5-7.9 (m, 5 H, aromatic), 6.0-6.5 (m, 4 H, vinylic), 3.18 (s, 3 H, O*Me*), 3.13 (s, 3 H, -O*Me*), 3.03 (s, 3 H, -O*Me*); IR (KBr, cm$^{-1}$) 3410, 2920, 1740, 1710, 1640, 1450; MS (neg. FAB) 1037 (M$^-$), 590, 445;

| | |
|---|---|
| Anal calcd. for $C_{57}H_{83}NO_{14}S \cdot H_2O$: | C 64.89, H 8.06, N 1.32 |
| Found: | C 64.03, H 8.21, N 1.20 |

Example 6.

42-Deoxy-42(S)-thiocyanato-rapamycin

Method A. Nucleophile used: 970 mg (10 mmol) of potassium thiocyanate; Reaction time: 24 h; Separation technique employed: Dynamax 2" silica column, eluent 40 % EtOAc/hexane, 20 mL/min; Yield of product as the monohydrate: 373 mg (39 %).

Spectral data follows : $^1$H NMR (400 MHz, DMSO-$d_6$) δ 6.0 - 6.5 (m, 4 H, vinylic), 4.5 (m, 1 H, *CH*SCN), 3.28 (s, 3 H, -O*Me*), 3.14 (s, 3 H, -O*Me*), 3.03 (s, 3 H, -O*Me*); IR (KBr, cm$^{-1}$) 3430, 2935, 1740, 1730, 1650, 1460; MS (neg. FAB) 1037 (M-), 590, 445; Anal calcd. for $C_{52}H_{78}N_2O_{12}S \cdot H_2O$: C 64.06, H 8.21, N 2.87

Found : C 64.62 , H 8.16, N 2.92

Example 7.

42-Deoxy-42(S)-(1,2;3,4-di-o-isopropylidene-galactopyranos-6-yl)rapamycin

Method A. Nucleophile used 1.0 g (4 mmol) of 1,2,3,4-di-isopropylidene-D-galactopyranose, Reaction time 48 h; Separation technique employed Dynamax 2" silica column eluent 35 , EtOAc/ Hexane 20 ml/min , Yield of product as the trihydrate: 243 mg (21 %)

Spectral data follows: $^1$H NMR (400 MHz, DMSO-$d_6$) δ 6.0 - 6.5 (m, 4 H, vinylic), 3.4-5.5 (m, 7 H, galactose protons), 3.25 (s, 3 H, O*Me*), 3.14 (s, 3 H, O*Me*), 3.04 (s, 3 H, O*Me*), 1.2-1.4 (4 s, 12 H, *Me*C(OR)$_2$); IR (KBr, cm$^{-1}$) 3420, 2920, 1720, 1640, 1450, 1370; MS (neg. FAB) 1155 (M-), 590, 563;

| | |
|---|---|
| Anal calcd. for $C_{63}H_{97}NO_{18} \cdot 3\ H_2O$: | C 62.53, H 8.52, N 1.16 |
| Found: | C 62.39, H 8.18, N 1.06. |

Example 8.

**42-Deoxy-42(S)-acetylamino-rapamycin**

Method A. Nucleophile used: 5 mL (100 mmol) of acetonitrile; Reaction time: 72 h; Separation technique employed: Dynamax 2" C-18 column, eluent 60 % acetonitrile/water, 20mL/min; Yield of product as the monohydrate: 360mg (30 %).

Spectral data follows: $^1$H NMR (400 MHz, DMSO-$d_6$) δ 7.67 (d, 1 H, CH*NH*Ac), 6.0 - 6.5 (m, 4H, vinylic), 3.23 (s, 3 H, -O*Me*), 3.4 (s, 3 H, -O*Me*), 3.03 (s, 3 H, -O*Me*), 91 (s, 3 H, NHCO*CH$_3$*); IR (KBr, cm$^{-1}$) 3400, 2910, 1715, 1640, 1530, 1450; MS (neg. FAB) 954 (M-), 590, 362;

| | |
|---|---|
| Anal calcd. for $C_{53}H_{82}N_2O_{13} \cdot H_2O$: | C 65.36, H 8.63, N 2.88 |
| Found: | C 65.15, H 8.31, N 3.07 |

Example 9.

**42-Deoxy-42(S)-cyanoamino-rapamycin**

Method A. Nucleophile used: 336 mg (8 mmol) of cyanamide; Reaction time: 48 h; Separation technique employed: Dynamax 2" silica column, eluent 45 % EtOAc/hexane, 20 mL/min; Yield of product: 310 mg (33 %).

Spectral data follows: $^1$H NMR (400 MHz, DMSO-$d_6$) δ 6.8 (d, 1 H, CH*NH*CN), 6.0 - 6.5 (m, 4 H, vinylic), 3.25 (s, 3 H, -O*Me*), 3.15 (s, 3 H, -O*Me*), 3.04 (s, 3 H, -O*Me*); IR (KBr, cm$^{-1}$) 3420, 2430, 2210, 1720, 1660, 1450; MS (neg. FAB) 937 (M-), 590, 345;

| | |
|---|---|
| Anal calcd. for $C_{52}H_{79}N_3O_{12}$: | C 66.64, H 8.39, N 4.48 |
| Found: | C 66 20, H 8.52, N 3.86 |

Example 10.

**42-Deoxy-42()-(6-hydroxymethyl-pyridin-2-yl-methoxy)-rapamycin**

Method B. Nucleophile used: 740 mg (6 mmol) of 2,6-bis(hydroxymethyl)pyridine; Reaction time: 72 h; Separation technique employed: Dynamax 2" C-18 column, eluent 65 % acetonitrile/water; 20 mL/min; Yield of product: 205 mg (20 %)

Spectral data follows: $^1$H NMR (400 MHz, DMSO-$d_6$) δ 7.8 (q, 1 H, arom), 7.27 (d, 1 H, arom), 7.24 (d, 1 H, arom), 6.0 - 6.5 (m, 4 H, vinylic), 4.5 (m, 4 H, O*CH$_2$*Pyr), 3.26 (s, 3 H, -O*Me*), 3.13 (s, 3 H, -O*Me*), 3.03 (s, 3 H, -O*Me*); IR (KBr, cm$^{-1}$) 3420, 2930,1750, 1720, 1640, 1450; MS (neg. FAB) 1034 (M-H), 590, 442;

| | |
|---|---|
| Anal calcd. for $C_{58}H_{86}N_2O_{14}$: | C 65.02, H 8.47, N 2.61 |
| Found: | C 65.14, H 8.77, N 2.25 |

Example 11.

<u>42-Deoxy-42()-[(Morpholine-4-carbonyl)-amino]-rapamycin</u>

Method A. Nucleophile used: 900 mg (8 mmol) of N-cyanomorpholine; Reaction time: 96 h; Separation technique employed: Dynamax 2" C-18 column, eluent 60 % acetonitrile/water, 20 mL/min; Yield of product as the monohydrate: 245 mg (24 %).

Spectral data follows: $^1$H NMR (400 MHz, DMSO-$d_6$) δ 6.0 - 6.5 (m, 4 H, vinylic), 5.97 (d, 1 H CH*NH*C=O), 3.53 (m 4 H, -*CH$_2$*O*CH$_2$*-), 3.25 (s 7 H , O*Me* and -*CH$_2$*N*CH$_2$*-), 14 (s, 3 H, -O*Me*), 3.04 (s, 3 H, -O*Me*); IR (KBr, cm$^{-1}$) 3400, 2930, 1730, 1640, 1460; MS (neg. FAB) 1025 (M-H), 590;

| | |
|---|---|
| Anal calcd. for $C_{56}H_{87}N_3O_{14} \cdot H_2O$: | C 64.43,.H 8.53, N 4.03 |
| Found: | C 64.13, H 8.46, N 3.88 |

Example 12.

<u>42-Deoxy-42()-(2,3-dihydroxy-propoxy)-rapamycin</u>

Method A. Nucleophile used: 920 mg (10 mmol) of glycerol; Reaction time: 96 h; 48 h at 35 °C; Separation technique employed: Dynamax 2" C-18 column, eluent 60 % acetonitrile/water, 20 mL/min; Yield of product: 208 mg (21 %).

Spectral data follows: $^1$H NMR (400 MHz, DMSO-$d_6$) δ 6.0 - 6.5 (m, 4 H, vinylic), 3.4 - 4.6 (m, 5 H, O*CH$_2$CH*(OH)*CH$_2$*OH), 3.25 (s, 3 H, -O*Me*), 3.14 (s, 3 H, -O*Me*), 3.04 (s, 3 H, -O*Me*); IR (KBr, cm$^{-1}$) 3420, 2930, 1730, 1650, 1450; MS (neg. FAB) 987 (M-), 590, 395;

| | |
|---|---|
| Anal calcd. for $C_{54}H_{85}NO_{15}$: | C 65.63, H 8.67, N 1.42 |
| Found: | C 65 63, H 8.89, N 1.33 |

Example 13.

**42-Deoxy-42()-(benzo[1,3]dioxol-5-ylmethoxy)-rapamycin**

To a solution of rapamycin (1.00 g, 1.10 mmol) in dichloromethane (6.0 mL) in a 25 mL flame dried round bottom flask equipped with a magnetic stirrer at room temperature was added 2,6-di-t-butyl-4-methylpyridine (986 mg, 4.80 mmol) portionwise. The reaction mixture was degassed, purged with nitrogen, and cooled to 0 °C. To the solution was added trifluoromethanesulfonic anhydride (0.20 mL, 1.19 mmol) dropwise over a period of 5 min. The solution became a cloudy white suspension. The reaction was stirred at 0 °C for 30 min, then warmed up to room temperature and stirred for 1 h. TLC analysis (50 % hexane/ethyl acetate) indicated formation of rapamycin triflate. To the reaction mixture is added 3,4-methylenedioxybenzyl alcohol (883 mg, 5.80 mmol). The reaction is let stirred under nitrogen at room temperature for 72 h. The reaction was quenched with saturated aqueous $NaHCO_3$ and the organic and aqueous layers were separated. The aqueous layer was extracted three times with ethyl acetate. The organic layers were combined, washed with brine, and dried over sodium sulfate. The solution was filtered and concentrated *in vacuo* to afford a pale yellow slush. TLC analysis (50 % hexane/ethyl acetate) indicated at least four compounds. The product mixture was separated by HPLC (40 % EtOAc/hexane, Dynamax 2" silica column, 20 mL/min) and four fractions A through D were collected. TLC analysis of fraction A indicated a mixture of two compounds. Further HPLC separation of fraction A (10 to 25 % EtOAc/hexane gradient over 280 min, Dynamax 2" silica column, 20 mL/min) afforded five fractions A/A though A/E. Spectroscopic analyses of fraction A/C (209 mg, 18 %) indicated the product to be the monohydrate of the title compound.

Spectral data follows: $^1$H NMR (400 MHz, DMSO-$d_6$) δ 6.78 - 6.85 (m, 3 H, aromatic), 6.42 (d, 1 H, C14 hydroxy), 6.10 - 6.39 (m, 4 H, vinylic), 5.95 (s. 2 H, -$CH_2$Ar), 5.48 (q, 1 H, C1*H*), 4.39 (s, 2 H, -O-$CH_2$-O-), 3.25 (s, 3 H, -O*Me*), 3.15 (s, 3 H, -O*Me*), 3.02 (s, 3 H, -O*Me*). IR (KBr, cm$^{-1}$) 3430, 2925, 1750, 1720, 1645, 1620, 1510, 1490, 1445. MS (neg. FAB): 1047 [M]$^-$, 590, 455, 321.

| | |
|---|---|
| Anal calcd. for $C_{59}H_{85}NO_{15}\cdot H_2O$: | C 66.47 , H 8.17 , N 1.31 |
| Found: | C 65.39 , H 8.17 , N 1.31 |

Example 14.

**31,42-Epi-rapamycin**

To a solution of rapamycin (829 mg, 0.90 mmole) in dichloromethane (3.0 mL) in a 10 mL flame dried round bottom flask equipped with a magnetic stirrer at room temperature was added 2,6-di-t-butyl-4-methyl pyridine (770 mg, 3.75 mmole) portionwise. The reaction mixture was degassed, purged with nitrogen, and cooled to 0 °C. To the solution was added trifluoromethanesulfonic anhydride (151 µL, 0.898 mmole) dropwise over a period of 3 min. The solution became cloudy. The reaction was stirred at 0 °C for 30 min, then warmed up to room temperature and stirred for another 30 min. TLC analysis (50 % hexane/ethyl acetate) indicated formation of rapamycin triflates. To the reaction mixture is added DMSO (1.0 mL) followed by water (0.51 mL, 2.83 mmole). The reaction is stirred under nitrogen at room temperature for 24 h, quenched with saturated aqueous $NaHCO_3$, and the organic and aqueous layers were separated. The aqueous layer was extracted three times with ethyl acetate. The organic layers were combined, washed with brine, and dried over sodium sulfate. The solution was filtered and concentrated *in vacuo* to afford a pale yellow foam. TLC analysis (50 % hexane/ethyl acetate) indicated two major compounds. The product mixture was separated by HPLC (60 % EtOAc/hexane, Dynamax 2" silica column, 20 mL/min), and two major fractions P and A were collected. Spectroscopic analyses of fraction P (121 mg, 15 %) indicated the product to be the monohydrate of the title compound.

Spectral data follows: $^1H$ NMR (400 MHz, $CDCl_3$) δ 5.90 - 6.35 (m, 5 H, vinylic), 5.48 (q, 1 H, C1*H*), 5.42 (d, 1 H, C29*H*), 5.25 (d, 1 H, C22*H*), 5.15 (m, 1 H, C25*H*), 4.52 (s, 1 H, C14 hydroxy), 3.68 (m, 1 H, C42*H*), 3.34 (s, 3 H, -O*Me*), 3.32 (s, 3 H, -O*Me*), 3.11 (s, 3 H, -O*Me*). IR (KBr, $cm^{-1}$): 3420, 2930, 1725, 1640, 1445. MS (neg. FAB): 913 $[M]^-$, 590, 321.

| | |
|---|---|
| Anal. calcd. for $C_{51}H_{79}NO_{13}\cdot H_2O$: | C 65.66 , H 8.69 , N 1.50 |
| Found: | C 65.33 , H 8.43 , N 1.38 |

Example 15

**42, 43-Didehydro-42-Deoxy-rapamycin**

Method A. Nucleophile used: 180 µL (10 mmol) of water and 800 mg (10 mmol) of dimethylsulfoxide. Reaction time: 48 h. Separation technique employed: Dynamax 2"

silica column, eluent 40% ethyl acetate:hexane, 20 mL/min. Yield of product: 135 mg (15%).

Spectral data follows: $^1$H NMR (400 MHz, DMSO-$d_6$) δ 6.0 - 6.5 (m, 5 H, vinylic), 5.7 (m, 1 H, olefinic), 3.8 (m, 1 H, MeOCHCH=CH), 3.25 (s, 3 H, -OMe), 3.15 (s, 3 H, -OMe), 3.04 (s, 3 H, -OMe), IR (KBr, cm$^{-1}$) 3430, 2940, 1720, 1650, 1450; MS (neg. FAB): 896 (M-), 590, 303.

| | |
|---|---|
| Anal calcd. for $C_{51}H_{77}NO_{12}$: | C 68.35, H 8.66, N 1.56 |
| Found: | C 68.11, H 8.61, N 1.44 |

Example 16.

**41-Demethoxy-42-dehydroxy-41-oxo-rapamycin**

Method A. Nucleophile used: 130 mg (5 mmol) of silver fluoride; Reaction time: 5 h; Separation technique employed: Dynamax 2" C-18 column, eluent 60 % acetonitrile/water, 20 mL/min; Yield of product: 238 mg (27 %).

Spectral data follows: $^1$H NMR (400 MHz, $CDCl_3$) : δ 5.9 - 6.5 (m, 4 H, vinylic), 3.34 (s, 3 H, -O*Me*), 3.13 (s, 3 H, -O*Me*); IR (KBr, cm$^{-1}$) 3420, 2925, 1725, 1640, 1450; MS (neg. FAB) 881 (M-H), 590;

| | |
|---|---|
| Anal calcd. for $C_{50}H_{75}NO_{12}$: | C 66.86, H 8.58, N 1.53 |
| Found: | C 66.28, H 8.69, N 1.72 |

Example 17.

**42-Deoxy-42(S)-(4,5-bis-methoxycarbonyl-[1,2,3]triazol-1-yl)-rapamycin**

To a solution of 42-deoxy-42-epi-azidorapamycin (470 mg, 0.5 mmol) was added dimethylacetylenedicarboxylate (75 mg, 0.53 mmol) in dichloromethane (5 mL). The solution was stirred at room temperature for 7 d. The solvent was removed *in vacuo* and the residue was subjected to normal phase HPLC (2 " Dynamax silica column, eluent 60 % ethyl acetate/hexane, 20 mL/min) to give after concentration *in vacuo* 221 mg (41 %) of triazole product as a monohydrate.

Spectral data follows: $^1$H NMR (400 MHz, DMSO-$d_6$) δ 6.0 - 6.5 (m, 4 H, vinylic), 5.18 (m, 1 H, MeOCHC*H*$N_3$), 3.90 (s, 3 H, *Me*$O_2$C-) 3.85 (s, 3 H, *Me*$O_2$C-) 3.16 (s, 3 H, -O*Me*), 3.13 (s, 3 H, -O*Me*), 3.04 (s, 3 H, -O*Me*) IR (KBr, cm$^{-1}$) 3440, 2940, 1740, 1650, 1450; MS (neg. FAB) 1081 (M-), 590, 446;

Anal calcd. for $C_{57}H_{84}N_4O_{16} \cdot H_2O$: C 62.24, H 7.92, N 5.09
Found: C 61.85, H 7.33, N 4.77

Example 18

42-Deoxy-42(S)-Chloro-rapamycin

Method A. Nucleophile used: 1.35 g (5 mmol) of tetrabutylammonium chloride; Reaction time: 48 h at 25 °C; Separation technique employed: 2" Dynamax silica column, eluent 45 % EtOAc/hexane, 20 mL/min; Yield of product: 354 mg (38 %)

Spectral data follows: $^1$H NMR (400 MHz, DMSO-$d_6$) δ 6.1 - 6.45 (m, 4 H, vinylic), 3.24 (s, 3 H, -O*Me*), 3.15 (s, 3 H, -O*Me*), 3.04 (s, 3 H, -O*Me*); IR (KBr, cm$^{-1}$) 3420, 2925, 1735, 1645, 1620, 1450; MS (neg. FAB) 931 (M-), 590, 339;
Anal calcd. for $C_{51}H_{78}NO_{12}Cl$: C 65.68, H 8.43, N 1.50
Found: C 65.61, H 8.62, N 1.28.

Example 19.

42-Deoxy-42(S){2-[2-(2-Methoxy-ethoxy)-ethoxy]-ethoxy}rapamycin

Method A. Nucleophile used: 820 mg (5 mmol) of triethyleneglycol monomethyl ether; Reaction time: 120 h at 25 °C; Separation technique employed: 2" Dynamax silica column, eluent 75 % EtOAc/hexane, 20 mL/min; Yield of product: 212 mg (20 %).

Spectral data follows: $^1$H NMR (400 MHz, DMSO-$d_6$) δ 6.1 - 6.45 (m, 4 H, vinylic), 3.49 (m, 12 H, -O$CH_2CH_2$O-), 3.27 (s, 3 H, -O*Me*), 3.24 (s, 3 H, -O*Me*), 3.14 (s, 3 H, -O*Me*), 3.04 (s, 3 H, -O*Me*); IR (KBr, cm$^{-1}$) 3425, 2925, 1720, 1645, 1450; MS (neg. FAB) 1059 (M-), 590, 467;
Anal calcd. for $C_{58}H_{93}NO_{16}$: C 65.70, H 8.84, N 1.32
Found: C 65.27, H 8.65, N 1.28

Example 20

42-Deoxy-42S-benzyloxy-rapamycin

To a solution of rapamycin (0.6319 g, 0.69 mmole) in dichloromethane (5.0 mL) in a round bottom flask (25 mL, flame dried) equipped with a magnetic stirrer at room

temperature was added 2,6-di-t-butyl-4-methyl pyridine (0.5372 g, 2.62 mmole) portionwise. The reaction mixture was degassed, purged with nitrogen, and cooled to 0 °C. To the solution was added trifluoromethanesulfonic anhydride (0.14 mL, 0.83 mmole) dropwise over a period of 3 minutes. The solution became a cloudy white suspension. The reaction was stirred at 0 °C for 30 minutes, then warmed up to room temperature and stirred for one hour. TLC analysis (50 % hexane/ethyl acetate) indicated complete formation of rapamycin-triflates. To the reaction mixture is added benzyl alcohol (0.80 mL, 7.73 mmole). The reaction mixture was stirred under nitrogen at room temperature for 48 hr. The reaction was quenched with saturated aqueous $NaHCO_3$, and the organic and aqueous layers were separated. The aqueous layer was extracted three times with ethyl acetate. The organic layers were combined, washed with brine, and dried over sodium sulfate. The solution was filtered and concentrated *in vacuo* to afford a pale yellow foam. TLC analysis (50% hexane/ethyl acetate) indicated at least three compounds. The product mixture was separated by HPLC (35 % EtOAc/hexane, Dynamax 2' silica column, 20 mL/min), and four fractions (A through D) were collected. TLC analysis of fraction A indicated a mixture of four compounds. Spectroscopic analyses of fraction B (0.150 g, 21.6 % overall yield) indicated that it was 42-deoxy-42S-benzyloxy-rapamycin.

Spectral data follows: $^1H$ NMR (400 MHz, DMSO) δ 7.25 - 7.35 (m, 5H, aromatic), 6.42 (s, 1H, hydroxy), 5.45 - 6.40 (m, 5H, vinylic), 4.48 (s, 2H, $-CH_2Ar$), 3.45 (s, 3H, $-OCH_3$), 3.18 (s, 3H, $-OCH_3$), 3.02 (s, 3H, $-OCH_3$); IR (film, $cm^{-1}$) : 3420, 2920, 1720, 1640, 1445; MS (neg. FAB) : 1003.5 $[M]^-$, 590.3, 411.2.

| | |
|---|---|
| Anal. calcd. for $C_{58}H_{85}NO_{13}$ : | C 68.86, H 8.53, N 1.39 |
| Found : | C 68.16, H 8.39, N 1.39 |

Example 21

**42-Deoxy-42S-O-[(pyridine-3-carbonyl)-amino]-rapamycin**

To a solution of rapamycin (0.6019 g, 0.66 mmole) in dichloromethane (5.0 mL) in a round bottom flask (25 mL, flame dried) equipped with a magnetic stirrer at room temperature was added 2,6-di-t-butyl-4-methyl pyridine (0.6140 g, 2.99 mmole) portionwise. The reaction mixture was degassed, purged with nitrogen, and cooled to 0 °C. To the solution was added trifluoromethanesulfonic anhydride (0.14 mL, 0.83 mmole) dropwise over a period of 5 minutes. The solution became a cloudy white suspension. The reaction was stirred at 0 °C for 30 minutes, then warmed up to room temperature and stirred for one hour. To the reaction mixture is added 3-cyanopyridine (0.6749 g, 6.48 mmole). The reaction was stirred under nitrogen at room temperature for

48 hr. The reaction was quenched with saturated aqueous $NaHCO_3$, and the organic and aqueous layers were separated. The aqueous layer was extracted three times with ethyl acetate. The organic layers were combined, washed with brine, and dried over sodium sulfate. The solution was filtered and concentrated *in vacuo* to afford a pale yellow powder. TLC analysis (ethyl acetate) indicated at least four compounds. The product mixture was separated by HPLC (40% 0.01 M $NH_4H_2PO_4$/acetonitrile, Dynamax 2' C18 column, 65 mL/min), and eighteen fractions (one through eighteen) were collected. TLC analysis of fractions seven through ten indicated they contained the desired product. The fractions were combined and extracted exhaustively with ethyl acetate. The organic layers were combined and concentrated *in vacuo* to afford a pale yellow powder. Spectroscopic analyses of the isolated compound (0.1563 g, 20.6 % overall yield) indicated that it was the ammonium phosphate salt, 42-Deoxy-42S-O-[(pyridine-3-carbonyl)-amino]-rapamycin dibasic ammonium phosphate salt (1:1).

Spectroscopic data follows: $^1$H NMR (400 MHz, DMSO) $\delta$ 7.9 - 9.5 (m, 4H, aromatic), 5.65 - 6.4 (m, 5H, vinylic), 3.15 (s, 3H, $-OCH_3$), 3.08 (s, 3H, $-OCH_3$), 3.02 (s, 3H, $-OCH_3$); IR (KBr, $cm^{-1}$) : 3440, 2925, 1715, 1635, 1450; MS (neg. FAB): 1148.0 ($[M-H]^-$), 590.3, 321.1.

| | |
|---|---|
| Anal. calcd. for $C_{57}H_{83}N_3O_{13}\bullet(NH_4)_2HPO_4$ | C 59.58, H 7.22, N 3.65 |
| Found : | C 58.63, H 6.73, N 3.42. |

## Pharmacology

Immunosuppressive activity was evaluated in an *in vitro* standard pharmacological test procedure to measure lymphocyte proliferation (LAF), in an *in vivo* procedure to evaluate the survival time of a pinch skin graft, and in an *in vivo* procedure to determine inhibition of T-cell mediated inflammatory response (adjuvant arthritis).

The comitogen-induced thymocyte proliferation procedure (LAF) was used as an *in vitro* measure of the immunosuppressive effects of representative compounds. Briefly, cells from the thymus of normal BALB/c mice are cultured for 72 hours with PHA and IL-1 and pulsed with tritiated thymidine during the last six hours. Cells are cultured with and without various concentrations of rapamycin, cyclosporin A, or test compound. Cells are harvested and incorporated; radioactivity is determined. Inhibition of lymphoproliferation is assessed in percent change in counts per minute from non-drug treated controls. The results are expressed by the following ratio, or as the percent inhibition of lymphoproliferation at 1 $\mu$M.

$$\frac{{}^3\text{H-control thymus cells} - \text{H}^3\text{-rapamycin-treated thymus cells}}{{}^3\text{H-control thymus cells} - \text{H}^3\text{-test compound-treated cells}}$$

The results for the rapamycin analog ($IC_{50}$analog) and rapamycin ($IC_{50}$rapa) as well as the ratio of the $ID_{50}$s of rapamycin to the analog (R/A) are given in the table below. A ratio less than 1.0 means the analog is less potent than rapamycin.

The *in vivo* test procedure is designed to determine the survival time of pinch skin graft from male DBA/2 donors transplanted to male BALB/c recipients. The method is adapted from Billingham R.E. and Medawar P.B., J. Exp. Biol. 28:385-402, (1951). Briefly, a pinch skin graft from the donor is grafted on the dorsum of the recipient as a homograft, and an autograft is used as control in the same region. The recipients are treated with either varying concentrations of cyclosporin A as test control or the test compound, intraperitoneally. Untreated recipients serve as rejection control. The graft is monitored daily and observations are recorded until the graft becomes dry and forms a blackened scab. This is considered as the rejection day. The mean graft survival time (MST -number of days $\pm$ S.D.) of the drug treatment group is compared with the control group. Rapamycin treatment provides a mean graft survival (MST) of 12.0$\pm$ 1.7 days.

The *in vivo* adjuvant arthritis standard pharmacological test procedure measures the ability of test compounds to prevent immune mediated inflammation and inhibit or treat rheumatoid arthritis. The following briefly describes the test procedure used. A group of rats (male inbred Wistar Lewis rats) are pre-treated with the compound to be tested (1h prior to antigen) and then injected with Freund's Complete Adjuvant (FCA) in the right hind paw to induce arthritis. The rats are then orally dosed on a Monday, Wednesday, Friday schedule from day 0-14 for a total of 7 doses. Both hind paws are measured on days 16, 23 and 30. The difference in paw volume (mL) from day 16 to day 0 is determined and a percent change from control is obtained. The left hind paw (uninjected paw) inflammation is caused by T-cell mediated inflammation and is recorded as percent change from control. The right hind paw inflammation, on the other hand, is caused by non-specific inflammation. Compounds were tested at a dose of 2 mg/kg. The results are expressed as the percent change in the uninjected paw at day 16 versus control; the more negative the percent change, the more potent the compound. Rapamycin provides between –70% and –90% change versus control, indicating that rapamycin treated rats have between 70-90% less immune induced inflammation than control rats.

The following table summarizes the results of the compounds of this invention in these three standard test procedures.

Table 1: Summary of Pharmacological Test Results

**Evaluation of Immunosuppressive Activity**

| | Adjuvant Arthritis | | LAF | Skin Graft | |
|---|---|---|---|---|---|
| Example | $IC_{50}$ analog | $IC_{50}$ rapa | R/A | MST ± S.D. | % Change |
| 2 | 2.00 | 0.4 | 0.16 | 7.2±0.4 | |
| 3 | 1.70 | 0.4 | 0.24 | 10.3±0.5 | |
| 4 | | 0.7 | 0.00 | | |
| 5 | 1.7 | 0.9 | 0.53 | 8.5±0.6 | |
| 6 | 5.2 | 0.9 | 0.17 | 7.0±0.0 | |
| 7 | 35 | 0.7 | 0.02 | | |
| 8 | 12.3 | 1.9 | 0.15 | 7.7±0.8 | –31 |
| 9 | 8.7 | 1.9 | 0.22 | 8.2±0.8 | |
| 10 | 27.93 | 0.5 | 0.02 | 8.0±0.9 | |
| 11 | 5.0 | 0.5 | 0.10 | 8.2±0.8 | –45 |
| 12 | 56.05 | 1.9 | 0.03 | | |
| 14 | 6.1 | 1.0 | 0.16 | | |
| 15 | 2.5 | 0.9 | 0.36 | 7.4±0.6 | |
| 16 | 35.77 | 0.9 | 0.03 | | |
| 17 | 27.03 | 0.7 | 0.03 | | |
| 18 | 3.2 | 0.8 | 0.26 | | |
| 19 | 14.99 | 1.0 | 0.07 | 7.7±0.5 | –34 |

The results of these standard pharmacological test procedures demonstrate immunosuppressive activity both in vitro and in vivo for the compounds of this invention. Positive ratios in the LAF test procedure indicate suppression of T cell proliferation. As transplanted pinch skin grafts are typically rejected with 6-7 days without the use of an immunosuppressive agent, the increased survival time of the skin graft when treated with the compounds of this invention further demonstrates their utility as immunosuppressive agents. The reduction of inflammatory joint swelling in the adjuvant rat model demonstrates their utility in the treatment of inflammatory diseases.

**Inhibition of growth of human tumors in vitro by rapamycin analogs**

Compounds of this invention which were tested for inhibition of several human tumor cell lines in the following assay procedure were found to inhibit prostate (PC-3, DU145), breast (T47D, SKBR-3), colon (MIP 101), ovarian (A2780S) tumor cells in submicromolar concentrations. The compounds of examples 3, 6, 7, 8, 9, and 17 inhibited prostate, breast, colon and ovarian cancer cells. The compound of example 4 was effective in inhibiting prostate, breast and ovarian cancer cells. The compound of

example 12 inhibited breast and ovarian cancer cells. The compound of example 17 was also effective in inhibiting leukemia (CCRF-CEM).

Human tumor cell lines were plated in 96-well plates (250 μL/well, 1-6 x $10^4$ cells/mL) in RPMI 1640 medium, containing 5% FBS (Fetal Bovine Serum). Twenty-four hours after plating, drugs were added at five log concentrations (0.01-100 μg/mL). After 48 hours exposure to drugs, cells were fixed with trichloroacteic acid, and stained with Sulforhodamine B. After washing with trichloroacetic acid, bound dye was solubilized in 10 mM Tris base and optical density (OD) was determined using a plate reader. Under conditions of the assay, the optical density is proportional to the number of cells in the well. $IC_{50}$s (concentrations causing 50% inhibition of cell growth) were determined from the growth inhibition plots. The assay is described in detail by Philip Skehan et al., J. National Cancer Institute **82**, 1107-1112, 1990.

Based on the results of these standard pharmacological test procedures, the compounds are useful in the treatment of transplantation rejection such as, heart, kidney, liver, bone marrow, and skin transplants; autoimmune diseases such as lupus, rheumatoid arthritis, diabetes mellitus, myasthenia gravis, and multiple sclerosis; and diseases of inflammation such as, psoriasis, dermatitis, eczema, seborrhea, inflammatory bowel disease and pulmonary inflammation such as asthma, chronic obstructive pulmonary disease, emphysema, bronchitis and the like; proliferative diseases such as restenosis following angioplasty procedures, fungal infections and in the treatment of certain tumors.

## Pharmaceutical Composition

The compounds may be administered neat or with a pharmaceutical carrier to a mammal in need thereof. The pharmaceutical carrier may be solid or liquid and the active compound shall be a therapeutically effective amount.

A solid carrier can include one or more substances which may also act as flavoring agents, lubricants, solubilizers, suspending agents, fillers, glidants, compression aids, binders or tablet-disintegrating agents; it can also be an encapsulating material. In powders, the carrier is a finely divided solid which is in admixture with the finely divided active ingredient. In tablets, the active ingredient is mixed with a carrier having the necessary compression properties in suitable proportions and compacted in the shape and size desired. The powders and tablets preferably contain up to 99% of the active ingredient. Suitable solid carriers include, for example, calcium phosphate, magnesium stearate, talc, sugars, lactose, dextrin, starch, gelatin, cellulose, methyl cellulose, sodium carboxymethyl cellulose, polyvinylpyrrolidine, low melting waxes and ion exchange resins.

Liquid carriers are used in preparing solutions, suspensions, emulsions, syrups, elixirs and pressurized compositions. The active ingredient can be dissolved or suspended in a pharmaceutically acceptable liquid carrier such as water, an organic solvent, a mixture of both or pharmaceutically acceptable oils or fats. The liquid carrier can contain other suitable pharmaceutical additives such as solubilizers, emulsifiers, buffers, preservatives, sweeteners, flavoring agents, suspending agents, thickening agents, colors, viscosity regulators, stabilizers or osmo-regulators. Suitable examples of liquid carriers for oral and parenteral administration include water (partially containing additives as above, e.g. cellulose derivatives, preferably sodium carboxymethyl cellulose solution), alcohols (including monohydric alcohols and polyhydric alcohols, e.g. glycols) and their derivatives, and oils (e.g. fractionated coconut oil and arachis oil). For parenteral administration, the carrier can also be an oily ester such as ethyl oleate and isopropyl myristate. Sterile liquid carriers are useful in sterile liquid form compositions for parenteral administration. The liquid carrier for pressurized compositions can be halogenated hydrocarbon or other pharmaceutically acceptable propellant.

Liquid pharmaceutical compositions which are sterile solutions or suspensions can be utilized by, for example, intramuscular, intraperitoneal or subcutaneous injection. Sterile solutions can also be administered intravenously. The compound can also be administered orally either in liquid or solid composition form. The formulated compound can further be administered intranasally through insufflation of a powder formulation, rectally or vaginally via suppositories, and topically or transdermally.

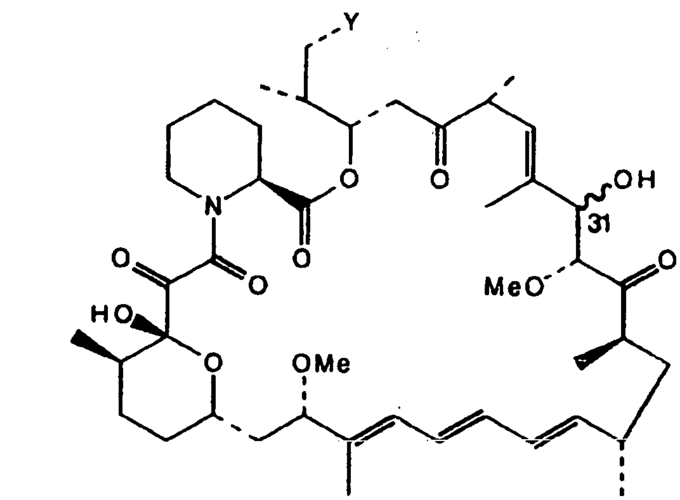
Furthermore, the formulated invention compound can be administered alone or in combination with one or more addidional immunoregluatory agents such as a corticosteroid, cyclophosphamide, rapamyucin, cyclosporin A, FK-506, OKT-3 or ATG as established by Stepkowski, Transplantation Proceedings 23: 507 (1991).

Preferably, the pharmaceutical composition is in unit dosage form, e.g. as tablets or capsules. In such form, the composition is sub-divided in unit doses containing appropriate quantities of the active ingredient; the unit dosage forms can be packaged compositions, for example, packeted powders, vials, ampoules, prefilled syringes or sachets containing liquids. The unit dosage form can be, for example, a capsule or tablet itself, or it can be the appropriate number of any such compositions in package form. The dosage to be used in the treatment must be subjectively determined by the attending physician.

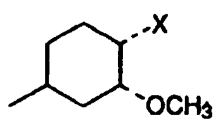
What is claimed:

-1-

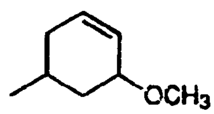
A compound of the formula



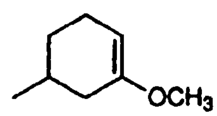
where Y is a group selected from



a

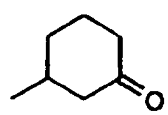


b ,



c

and



d

and X is selected from hydroxy, $-OR^1$, $-SO_2Ar$, $-SO_2R^1$, $N_3$, -OAr, -NH(C=O)Ar, $-NH(C=O)R^1$, $-NH(C=O)NR^2R^3$, -NHCN, I, Cl, F, Br, -SCN, or 1,2,3-triazole optionally substituted with methoxycarbonyl;

wherein $R^1$ is $C_1$ to $C_{10}$ alkyl, cycloalkyl of 3 to 10 carbon atoms, $-(CH_2)_{1-10}NHR^2$, piperidinyl, pyrrolidinyl, piperazinyl, $-(CH_2)_{1-10}Ar$, $-CH_2CH(OR^4)CH_2OR^5$, or $-CH_2$-di- 1,2:3,4-diisopropylidenegalactose;

$R^2$ and $R^3$ are independently $C_1$ to $C_{10}$ alkyl, Ar, H, or $-(CH_2)_{1-10}Ar$;

$R^4$ and $R^5$ are independently H, $C_1$ to $C_{10}$ alkyl, $-(CH_2)_{1-10}Ar$, or $R^4$ and $R^5$ together form isopropylidene; and

Ar is selected independently from phenyl, naphthyl, pyridyl, quinolyl, indolyl, imidazolyl, triazoly, tetrazolyl, furanyl, and Ar may be substituted with one or two substituents selected independently from F, Cl, Br, I, $NO_2$, OH, $C_1$–$C_{10}$ alkyl, $C_1$–$C_{10}$ alkoxy, and hydroxymethyl; or Ar is 3,4-methylenedioxyphenyl;

or a pharmaceutically acceptable salt thereof when one can be formed.

-2-

A compound according to claim 1 which is 42-deoxy-42(S)-iodo-rapamycin.

-3-

A compound according to claim 1 which is 42-deoxy-42(S)-azido-rapamycin.

-4-

A compound according to claim 1 which is 42-deoxy-42-0-(2,2-dimethyl-[1,3]-dioxolan-4-ylmethyl)rapamycin.

-5-

A compound according to claim 1 which is 42-deoxy-42(S)-benzenesulfonyl-rapamycin.

-6-

A compound according to claim 1 which is 42-deoxy-42(S)-thiocyanato-rapamycin.

-7-

A compound according to claim 1 which is 42-deoxy-42(S)-(1,2:3,4-di-o-isopropylidene-galactopyranos-6-yl)rapamycin.

-8-

A compound according to claim 1 which is 42-deoxy-42(S)-acetylamino-rapamycin.

-9-

A compound according to claim 1 which is 42-deoxy-42(S)-cyanoamino-rapamycin.

-10-

A compound according to claim 1 which is 42-deoxy-42(S)-(6-hydroxymethyl-pyridin-2-yl-methoxy)-rapamycin.

-11-

A compound according to claim 1 which is 42-deoxy-42(S)-[(morpholine-4-carbonyl)-amino]-rapamycin.

-12-

A compound according to claim 1 which is 42-deoxy-42(S)-(2,3-dihydroxy-propoxy)-rapamycin.

-13-

A compound according to claim 1 which is 42-deoxy-42(S)-(benzo[1,3]dioxol-5-ylmethoxy)-rapamycin.

-14-

A compound according to claim 1 which is 31,42-epi-rapamycin.

-15-

A compound according to claim 1 which is 42, 43-didehydro-42-deoxy-rapamycin.

-16-

A compound according to claim 1 which is 41-demethoxy-42-dehydroxy-41-oxo-rapamycin.

-17-

A compound according to claim 1 which is 42-deoxy-42-(4,5-bis-methoxycarbonyl-[1,2,3]triazol-1-yl)-rapamycin.

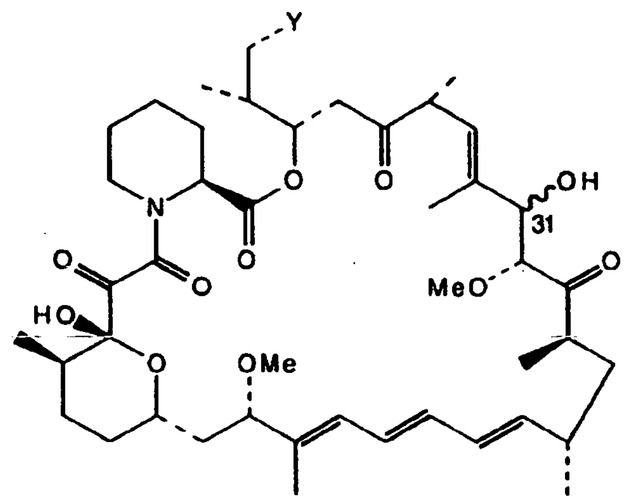
-18-

A compound according to claim 1 which is 42-deoxy-42(S)-Chloro-rapamycin.

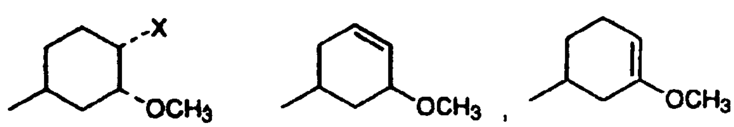
-19-

A compound according to claim 1 which is 42-deoxy-42(S)-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethoxy)-rapamycin.

A method of treating transplantation rejection or host vs. graft disease in a mammal by administering thereto an immunosuppressing effective amount of a compound having the formula

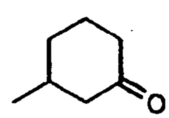


where Y is a group selected from



a b c

and



d

and X is selected from hydroxy, $-OR^1$, $-SO_2Ar$, $-SO_2R^1$, $N_3$, -OAr, -NH(C=O)Ar, $-NH(C=O)R^1$, $-NH(C=O)NR^2R^3$, -NHCN, I, Cl, F, Br, -SCN, or 1,2,3-triazole optionally substituted with methoxycarbonyl;

wherein $R^1$ is $C_1$ to $C_{10}$ alkyl, cycloalkyl of 3 to 10 carbon atoms, $-(CH_2)_{1-10}NHR^2$, piperidinyl, pyrrolidinyl, piperazinyl, $-(CH_2)_{1-10}Ar$, $-CH_2CH(OR^4)CH_2OR^5$, or $-CH_2$-di- 1,2:3,4-diisopropylidenegalactose;

$R^2$ and $R^3$ are independently $C_1$ to $C_{10}$ alkyl, Ar, H, or $-(CH_2)_{1-10}Ar$;

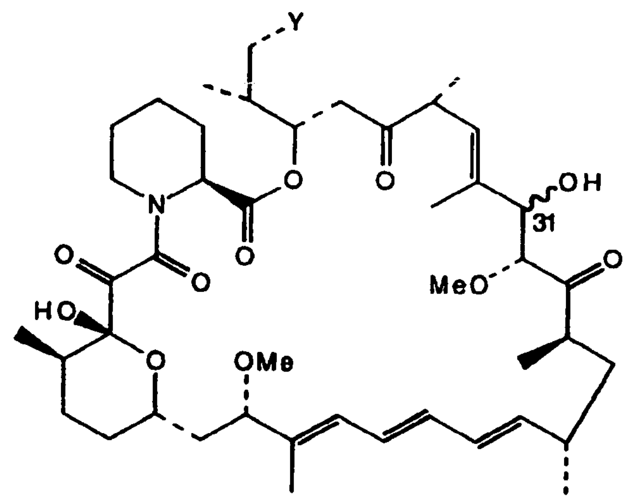
$R^4$ and $R^5$ are independently H, $C_1$ to $C_{10}$ alkyl, $-(CH_2)_{1-10}Ar$, or $R^4$ and $R^5$ together form isopropylidene; and

Ar is selected independently from phenyl, naphthyl, pyridyl, quinolyl, indolyl, imidazolyl, triazoly, tetrazolyl, furanyl, and Ar may be substituted with one or two substituents selected independently from F, Cl, Br, I, $NO_2$, OH, $C_1$–$C_{10}$ alkyl, $C_1$–$C_{10}$ alkoxy, and hydroxymethyl; or Ar is 3,4-methylenedioxyphenyl;

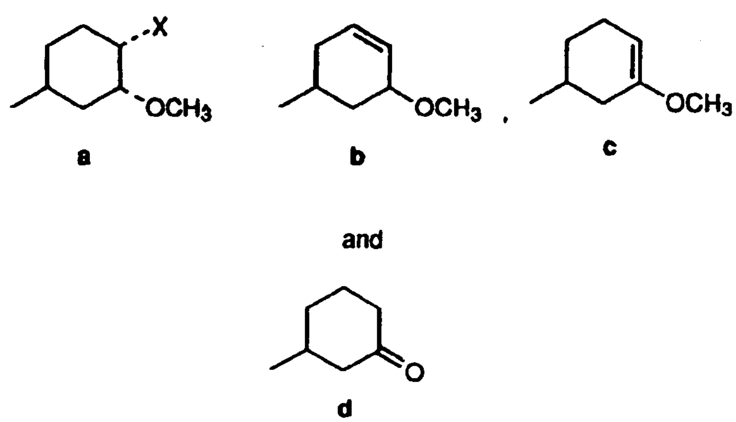
or a pharmaceutically acceptable salt thereof when one can be formed.

-21-

A method of treating asthma in a mammal by administering thereto an asthma inhibiting effective amount of a compound having the formula




where Y is a group selected from



and X is selected from hydroxy, $-OR^1$, $-SO_2Ar$, $-SO_2R^1$, $N_3$, -OAr, -NH(C=O)Ar, $-NH(C=O)R^1$, $-NH(C=O)NR^2R^3$, -NHCN, I, Cl, F, Br, -SCN, or 1,2,3-triazole optionally substituted with methoxycarbonyl;

wherein $R^1$ is $C_1$ to $C_{10}$ alkyl, cycloalkyl of 3 to 10 carbon atoms, $-(CH_2)_{1-10}NHR^2$, piperidinyl, pyrrolidinyl, piperazinyl, $-(CH_2)_{1-10}Ar$, $-CH_2CH(OR^4)CH_2OR^5$, or $-CH_2$-di- 1,2:3,4-diisopropylidenegalactose;

$R^2$ and $R^3$ are independently $C_1$ to $C_{10}$ alkyl, Ar, H, or $-(CH_2)_{1-10}Ar$;

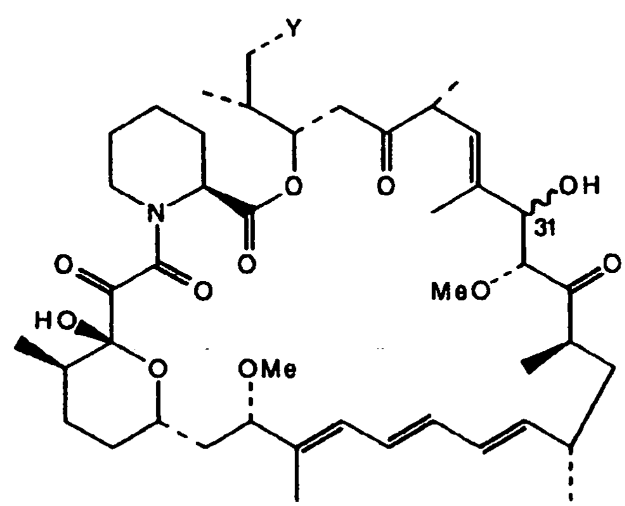
$R^4$ and $R^5$ are independently H, $C_1$ to $C_{10}$ alkyl, $-(CH_2)_{1-10}Ar$, or $R^4$ and $R^5$ together form isopropylidene; and

Ar is selected independently from phenyl, naphthyl, pyridyl, quinolyl, indolyl, imidazolyl, triazoly, tetrazolyl, furanyl, and Ar may be substituted with one or two substituents selected independently from F, Cl, Br, I, $NO_2$, OH, $C_1$–$C_{10}$ alkyl, $C_1$–$C_{10}$ alkoxy, and hydroxymethyl; or Ar is 3,4-methylenedioxyphenyl;

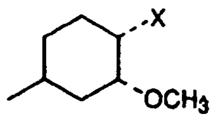
or a pharmaceutically acceptable salt thereof when one can be formed.

-22-

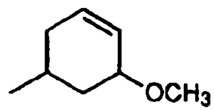
A method of treating rheumatoid arthritis in a mammal by administering thereto an arthritis inhibiting effective amount of a compound having the formula



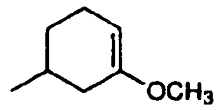

where Y is a group selected from



a

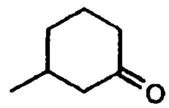


b ,



c

and



d

and X is selected from hydroxy, $-OR^1$, $-SO_2Ar$, $-SO_2R^1$, $N_3$, -OAr, -NH(C=O)Ar, $-NH(C=O)R^1$, $-NH(C=O)NR^2R^3$, -NHCN, I, Cl, F, Br, -SCN, or 1,2,3-triazole optionally substituted with methoxycarbonyl;

wherein $R^1$ is $C_1$ to $C_{10}$ alkyl, cycloalkyl of 3 to 10 carbon atoms, $-(CH_2)_{1\text{-}10}NHR^2$, piperidinyl, pyrrolidinyl, piperazinyl, $-(CH_2)_{1\text{-}10}Ar$, $-CH_2CH(OR^4)CH_2OR^5$, or $-CH_2$-di- 1,2:3,4-diisopropylidenegalactose;

$R^2$ and $R^3$ are independently $C_1$ to $C_{10}$ alkyl, Ar, H, or $-(CH_2)_{1\text{-}10}Ar$;

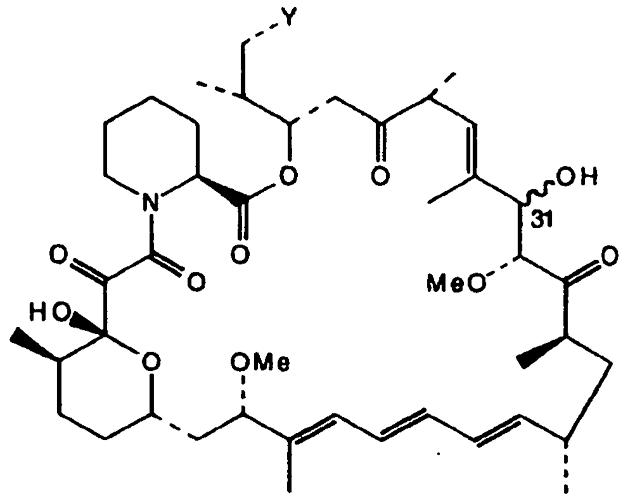
$R^4$ and $R^5$ are independently H, $C_1$ to $C_{10}$ alkyl, $-(CH_2)_{1\text{-}10}Ar$, or $R^4$ and $R^5$ together form isopropylidene; and

Ar is selected independently from phenyl, naphthyl, pyridyl, quinolyl, indolyl, imidazolyl, triazoly, tetrazolyl, furanyl, and Ar may be substituted with one or two substituents selected independently from F, Cl, Br, I, $NO_2$, OH, $C_1$–$C_{10}$ alkyl, $C_1$–$C_{10}$ alkoxy, and hydroxymethyl; or Ar is 3,4-methylenedioxyphenyl;

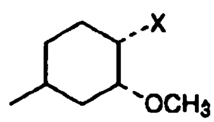
or a pharmaceutically acceptable salt thereof when one can be formed.

-23-

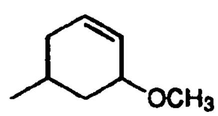
A method of treating fungal infections in a mammal by administering thereto an antifungal effective amount of a compound having the formula



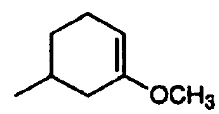
where Y is a group selected from



a

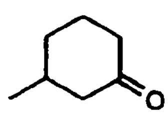


b ,



c

and



d

and X is selected from hydroxy, $-OR^1$, $-SO_2Ar$, $-SO_2R^1$, $N_3$, $-OAr$, $-NH(C{=}O)Ar$, $-NH(C{=}O)R^1$, $-NH(C{=}O)NR^2R^3$, -NHCN, I, Cl, F, Br, -SCN, or 1,2,3-triazole optionally substituted with methoxycarbonyl;

wherein $R^1$ is $C_1$ to $C_{10}$ alkyl, cycloalkyl of 3 to 10 carbon atoms, $-(CH_2)_{1\text{-}10}NHR^2$, piperidinyl, pyrrolidinyl, piperazinyl, $-(CH_2)_{1\text{-}10}Ar$, $-CH_2CH(OR^4)CH_2OR^5$, or $-CH_2$-di- 1,2:3,4-diisopropylidenegalactose;

$R^2$ and $R^3$ are independently $C_1$ to $C_{10}$ alkyl, Ar, H, or $-(CH_2)_{1\text{-}10}Ar$;

$R^4$ and $R^5$ are independently H, $C_1$ to $C_{10}$ alkyl, $-(CH_2)_{1\text{-}10}Ar$, or $R^4$ and $R^5$ together form isopropylidene; and

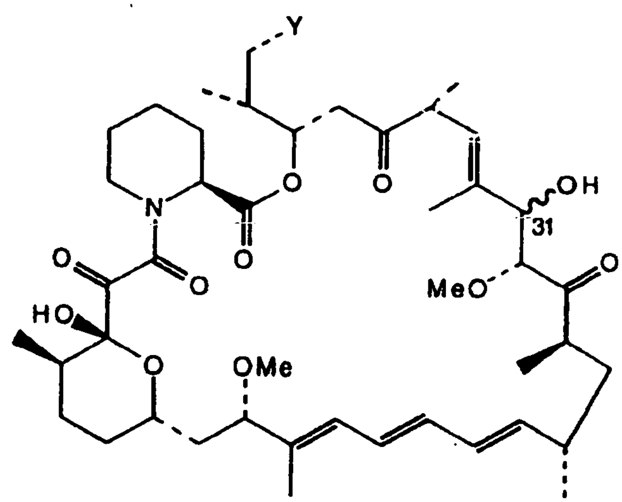
Ar is selected independently from phenyl, naphthyl, pyridyl, quinolyl, indolyl, imidazolyl, triazoly, tetrazolyl, furanyl, and Ar may be substituted with one or

two substituents selected independently from F, Cl, Br, I, $NO_2$, OH, $C_1$–$C_{10}$ alkyl, $C_1$–$C_{10}$ alkoxy, and hydroxymethyl; or Ar is 3,4-methylenedioxyphenyl;

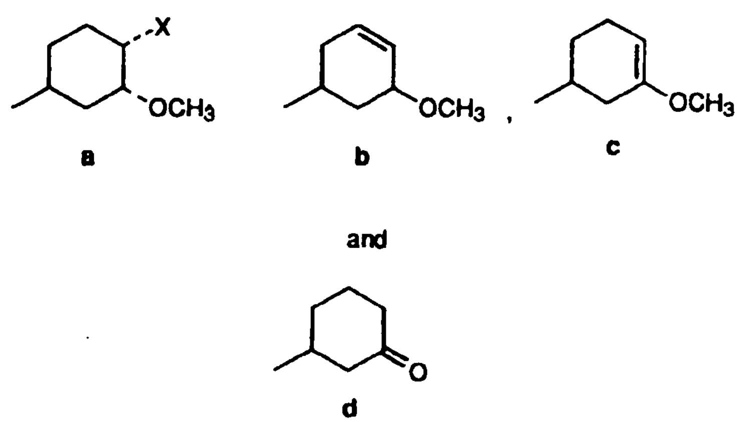
or a pharmaceutically acceptable salt thereof when one can be formed.

-24-

A method of inhibiting restenosis in a mammal by administering thereto a restenosis inhibiting effective amount of a compound having the formula



where Y is a group selected from



and X is selected from hydroxy, $-OR^1$, $-SO_2Ar$, $-SO_2R^1$, $N_3$, -OAr, -NH(C=O)Ar, $-NH(C=O)R^1$, $-NH(C=O)NR^2R^3$, -NHCN, I, Cl, F, Br, -SCN, or 1,2,3-triazole optionally substituted with methoxycarbonyl;

wherein $R^1$ is $C_1$ to $C_{10}$ alkyl, cycloalkyl of 3 to 10 carbon atoms, $-(CH_2)_{1-10}NHR^2$, piperidinyl, pyrrolidinyl, piperazinyl, $-(CH_2)_{1-10}Ar$, $-CH_2CH(OR^4)CH_2OR^5$, or $-CH_2$-di- 1,2:3,4-diisopropylidenegalactose;

$R^2$ and $R^3$ are independently $C_1$ to $C_{10}$ alkyl, Ar, H, or $-(CH_2)_{1-10}Ar$;

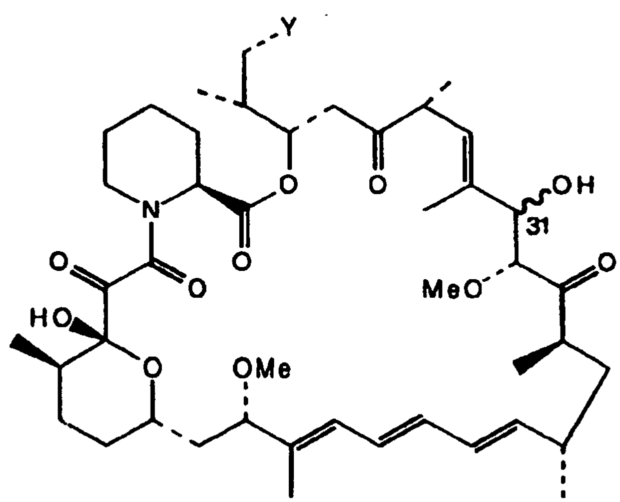
$R^4$ and $R^5$ are independently H, $C_1$ to $C_{10}$ alkyl, $-(CH_2)_{1-10}Ar$, or $R^4$ and $R^5$ together form isopropylidene; and

Ar is selected independently from phenyl, naphthyl, pyridyl, quinolyl, indolyl, imidazolyl, triazoly, tetrazolyl, furanyl, and Ar may be substituted with one or two substituents selected independently from F, Cl, Br, I, $NO_2$, OH, $C_1$–$C_{10}$ alkyl, $C_1$–$C_{10}$ alkoxy, and hydroxymethyl; or Ar is 3,4-methylenedioxyphenyl;

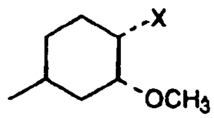
or a pharmaceutically acceptable salt thereof when one can be formed.

-25-

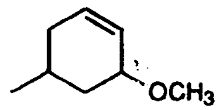
A method of treating tumors which comprises administration to a mammal having a tumor a tumor-inhibiting effective amount of a compound of the formula




where Y is a group selected from



a



b

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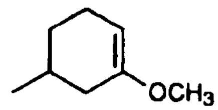
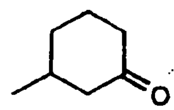


c

and



d

and X is selected from hydroxy, $-OR^1$, $-SO_2Ar$, $-SO_2R^1$, $N_3$, -OAr, -NH(C=O)Ar, $-NH(C=O)R^1$, $-NH(C=O)NR^2R^3$, -NHCN, I, Cl, F, Br, -SCN, or 1,2,3-triazole optionally substituted with methoxycarbonyl;

wherein $R^1$ is $C_1$ to $C_{10}$ alkyl, cycloalkyl of 3 to 10 carbon atoms, $-(CH_2)_{1-10}NHR^2$, piperidinyl, pyrrolidinyl, piperazinyl, $-(CH_2)_{1-10}Ar$, $-CH_2CH(OR^4)CH_2OR^5$, or $-CH_2$-di- 1,2:3,4-diisopropylidenegalactose;

$R^2$ and $R^3$ are independently $C_1$ to $C_{10}$ alkyl, Ar, H, or $-(CH_2)_{1-10}Ar$;

$R^4$ and $R^5$ are independently H, $C_1$ to $C_{10}$ alkyl, $-(CH_2)_{1-10}Ar$, or $R^4$ and $R^5$ together form isopropylidene; and

Ar is selected independently from phenyl, naphthyl, pyridyl, quinolyl, indolyl, imidazolyl, triazoly, tetrazolyl, furanyl, and Ar may be substituted with one or two substituents selected independently from F, Cl, Br, I, $NO_2$, OH, $C_1$–$C_{10}$ alkyl, $C_1$–$C_{10}$ alkoxy, and hydroxymethyl; or Ar is 3,4-methylenedioxyphenyl;

or a pharmaceutically acceptable salt thereof when one can be formed.

-26-

The method according to claim 25 wherein the tumor inhibited is leukemia or prostate, breast, colon, or ovarian tumor.

-27-

The method according to claim 25 wherein the tumor inhibiting compound is selected from:

42-deoxy-42(S)-azido-rapamycin,

42-deoxy-42(S)-O-(2,2-dimethyl-[1,3]-dioxolan-4-ylmethyl)rapamycin,

42-deoxy-42(S-)benzenesulfonyl-rapamycin,

42-deoxy-42(S)-thiocyanato-rapamycin,

42-deoxy-42(S)-(1,2:3,4-di-o-isopropylidene-galactopyranos-6-yl)rapamycin,

42-deoxy-42(S)-acetylamino-rapamycin

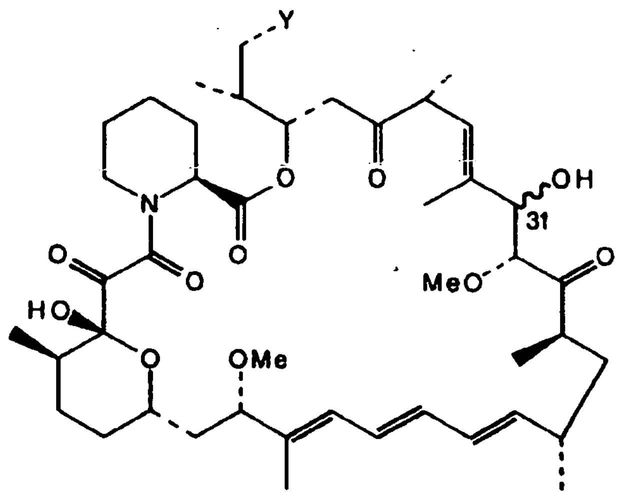
42-deoxy-42(S)-cyanoamino-rapamycin,

42-deoxy-42(S)-(2,3-dihydroxy-propoxy)-rapamycin or

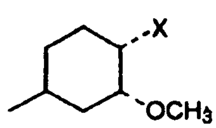
42-deoxy-42(S)-(4,5-bis-methoxycarbonyl-[1,2,3]triazol-1-yl)-rapamycin.

-28-

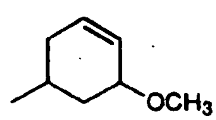
A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of a compound of the formula




where Y is a group selected from




a

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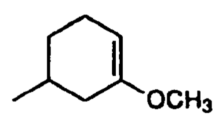
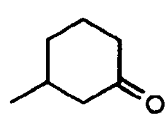



c

and




d

and X is selected from hydroxy, $-OR^1$, $-SO_2Ar$, $-SO_2R^1$, $N_3$, -OAr, -NH(C=O)Ar, $-NH(C{=}O)R^1$, $-NH(C{=}O)NR^2R^3$, -NHCN, I, Cl, F, Br, -SCN, or 1,2,3-triaz le optionally substituted with methoxycarbonyl;

wherein $R^1$ is $C_1$ to $C_{10}$ alkyl, cycloalkyl of 3 to 10 carbon atoms, $-(CH_2)_{1-10}NHR^2$, piperidinyl, pyrrolidinyl, piperazinyl, $-(CH_2)_{1-10}Ar$, $-CH_2CH(OR^4)CH_2OR^5$, or $-CH_2$-di- 1,2:3,4-diisopropylidenegalactose;

$R^2$ and $R^3$ are independently $C_1$ to $C_{10}$ alkyl, Ar, H, or $-(CH_2)_{1-10}Ar$;

$R^4$ and $R^5$ are independently H, $C_1$ to $C_{10}$ alkyl, $-(CH_2)_{1-10}Ar$, or $R^4$ and $R^5$ together form isopropylidene; and

Ar is selected independently from phenyl, naphthyl, pyridyl, quinolyl, indolyl, imidazolyl, triazoly, tetrazolyl, furanyl, and Ar may be substituted with one or two substituents selected independently from F, Cl, Br, I, $NO_2$, OH, $C_1$–$C_{10}$ alkyl, $C_1$–$C_{10}$ alkoxy, and hydroxymethyl; or Ar is 3,4-methylenedioxyphenyl;

or a pharmaceutically acceptable salt thereof when one can be formed.

# INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/15438

**A. CLASSIFICATION OF SUBJECT MATTER**
IPC 6 C07D498/18 A61K31/445

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X,Y | WO 95 14023 A (ABBOTT LAB) 26 May 1995<br>see the whole document | 1-28 |
| Y | US 5 525 610 A (CAUFIELD CRAIG E ET AL)<br>11 June 1996<br>see the whole document | 1-28 |
| Y | US 5 164 399 A (FAILLI AMEDEO A ET AL) 17<br>November 1992<br>see the whole document | 1-28 |

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 18 December 1997 | 30.01.98 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax: (+31-70) 340-3016 | Steendijk, M |

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Information on patent family members

International Application No
PCT/US 97/15438

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| | | EP 0729471 A | 04-09-96 |
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